3831.03 <u>PATENT</u>

Serial No.: 10/626,459

IN THE CLAIMS

Claims 1-37 (Canceled)

38. (New) A method for treatment of cartilage lesions or defects by providing an implantable construct comprising activated chondrocytes or stem cells that could be differentiated into chondrocytes and further providing means for formation of a superficial cartilage layer, said method comprising steps:

- a) isolating autologous or heterologous chondrocytes or providing cells that could be differentiated into chondrocytes;
- b) expanding said isolated chondrocytes or cells in a growth medium;
- c) suspending said isolated expanded chondrocytes or cells in a collagen-containing solution;
- d) providing a three-dimensional support matrix containing plurality of pores;
- e)incorporating a suspension obtained in step c) into said support matrix thereby producing a seeded support matrix;
- f) preparing an implantable construct for implantation into said cartilage lesion by activating said chondrocytes or cells by subjecting said seeded support matrix to conditions promoting activation and propagation of said chondrocytes or cells within said support matrix, wherein said conditions for activation and propagation of chondrocytes comprise

perfusing said seeded support matrix with a culture medium at a flow rate from about 1 $\mu L/min$ to about 500 $\mu L/min$ and

applying to said seeded support matrix of step e) a cyclic hydrostatic pressure from about 0.01 MPa to about 10 MPa above atmospheric pressure at about 0.01 to about 1 Hz for from about 1 to about 8 hours followed by applying a static atmospheric pressure

3831.03 <u>PATENT</u>

Serial No.: 10/626,459

for from 16 to about 23 hours, said protocol repeated for from about one day to about ninety days;

- g) implanting said construct into said cartilage lesion; and
- h) depositing polyethylene glycol cross-linked with methylated collagen over said construct,

wherein said deposition of said polyethylene glycol crosslinked with methylated collagen over said implanted construct results within three months in formation of the superficial cartilage layer that overgrows said construct implanted within said lesion.

39. (New) The method of claim 38 further comprising step i) wherein a bottom tissue sealant is deposited into said cartilage lesion before said construct is implanted therein,

wherein said bottom sealant is selected from the group consisting of gelatin, a copolymer of polyethylene glycol and polylactide or poly-glycolide, periodate-oxidized gelatin, 4-armed pentaerythritol thiol and a polyethylene glycol diacrylate, 4-armed tetra-succinimidyl ester or tetra-thiol derivatized PEG, photopolymerizable polyethylene glycol-co-poly(α-hydroxy acid) diacrylate macromer, 4-armed polyethylene glycol derivatized with succinimidyl ester and thiol further cross-linked with methylated collagen, derivatized polyethylene glycol (PEG), polyethylene glycol cross-linked with alkylated collagen, (PEG) hydrosuccinimidyl or tetra-thiol derivatized PEG, PEG cross-linked with methylated collagen, and a combination thereof.

40. (New) The method of claim 38 wherein said support matrix is a sponge, scaffold, honeycomb or honeycomb-like lattice prepared from a compound selected from the group consisting of a Type I

3831.03 <u>PATENT</u>

Serial No.: 10/626,459

collagen, Type II collagen, Type IV collagen, gelatin, agarose, cell-contracted collagen containing proteoglycans, glycosaminoglycans or glycoproteins.

- 41. (New) The method of claim 40 wherein said support matrix is a prepared from Type I collagen.
- 42. (New) The method of claim 38 wherein said hydrostatic cyclic pressure is applied from about 0.05 MPa to about 3 MPa at 0.1 to about 0.5 Hz.
- 43. (New) The method of claim 38 wherein said perfusion flow rate is from about $5\,\mu\text{L/min}$ to about $50\,\mu\text{L/min}$.
- 44. (New) The method of claim 43 wherein said perfusion flow rate is about $5\,\mu\mathrm{L/min}$.
- 45. (New) The method of claim 38 wherein cell activation is performed under oxygen concentration from about 1% to about 20%.
- 46. (New) The method of claim 45 wherein cell activation is performed under oxygen concentration from about 2% to about 5%.
- 47. (New) The method of claim 38 wherein said superficial cartilage layer grows into or provides the same type of surface as a synovial membrane of the intact joint.
- 48. (New) The method of claim 38 wherein said construct is prepared in vitro, ex vivo or in vivo.